Cell, Volume *134*

Supplemental Data

A Self-Associating Protein Critical for Chromosome Attachment, Division, and Polar Organization in *Caulobacter*

Gitte Ebersbach, Ariane Briegel, Grant J. Jensen, and Christine Jacobs-Wagner

Supplemental Figures

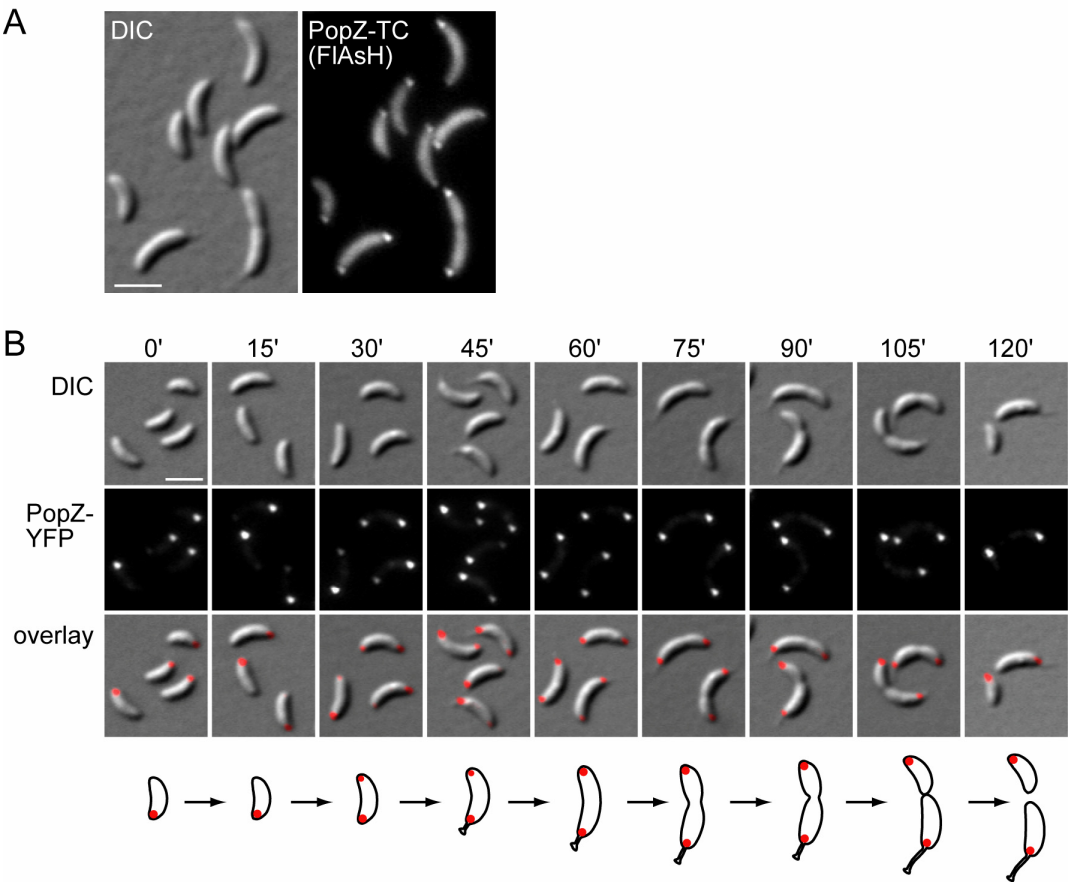

Figure S1: Polar localization of PopZ during the cell cycle

(A) PopZ polarly localizes. Microscopy of functional PopZ-TC expressed as the only copy from the native *popZ* locus. Cells of strain CB15N *popZ*::*pBGent-PopZ-TC* stained with FlAsH for 20 min before microscopy.

(B) Time-course microscopy of cells expressing *popZ-yfp* as the only copy of *popZ* from the native locus (CB15N *popZ*::*pBGent-PopZ-YFP*). Swarmer cells were isolated from a log phase M2G culture and resuspended in fresh M2G medium to allow growth to resume in a cell cycle synchronous fashion. Samples were collected for microscopy every 15 min until one cell cycle was completed. PopZ-YFP localizes to the old pole of swarmer cells. Subsequently, a second focus

appears at the new pole. Division yields daughter cells inheriting PopZ at their old pole. Scale bar, 2 μm.

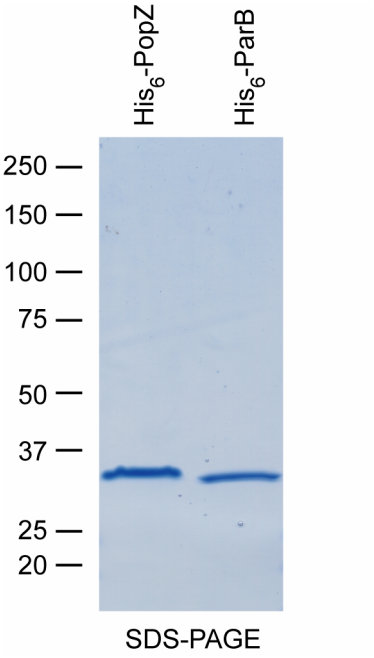

Figure S2. SDS-PAGE of purified His_6-PopZ and His_6-ParB.

One μg of each protein was loaded onto a 4-15% gradient gel (BioRad).

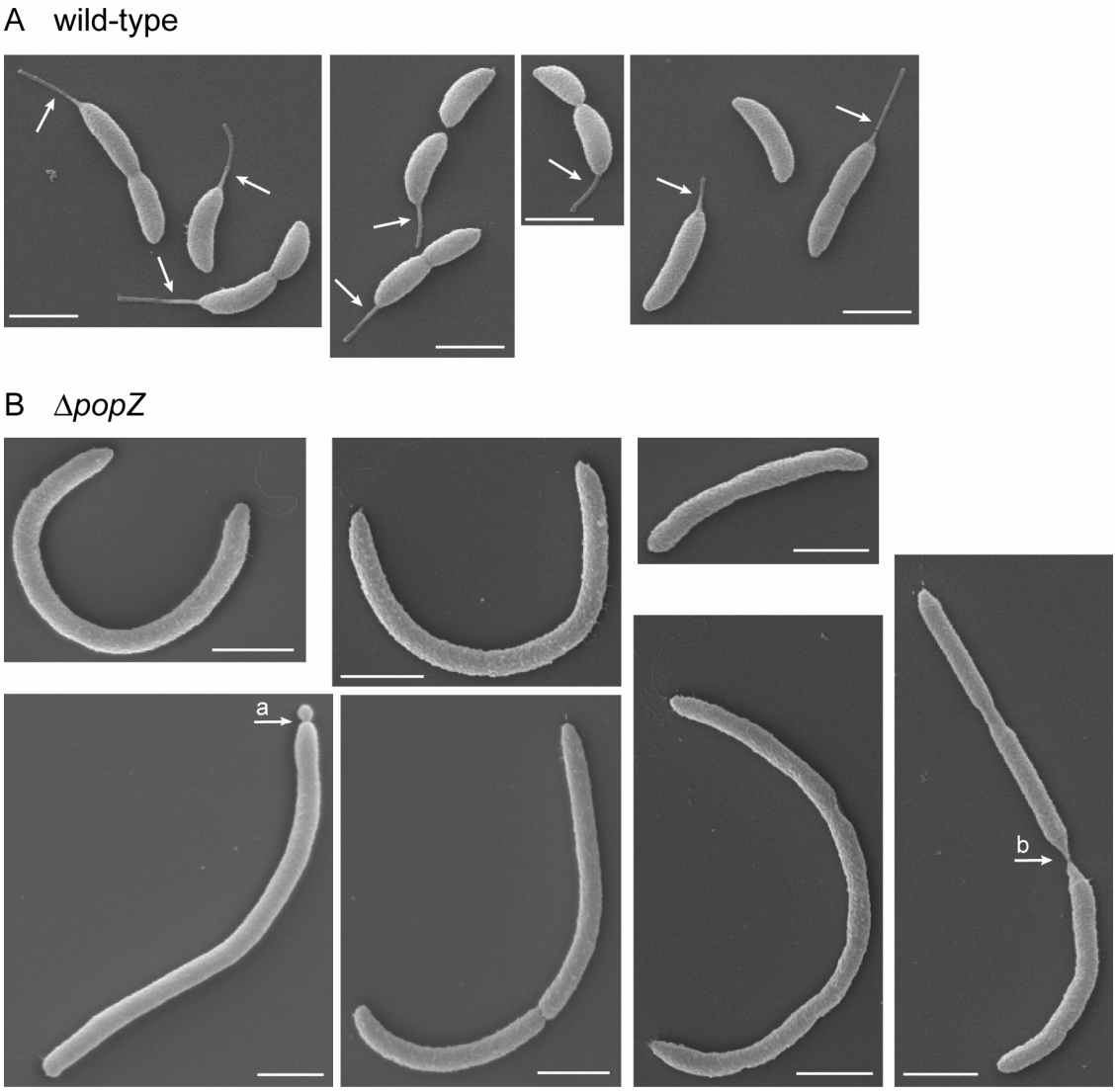

Figure S3. PopZ is involved in cell division and polar stalk morphogenesis.

(A) Scanning electron microscopy (SEM) images of fixed cells of strain CB15N. White arrows show stalks.

(B) SEM of fixed cells of strain CB15N Δ*popZ*. White arrow 'a' shows a cell dividing near one pole leading to the formation of a minicell. White arrow 'b' shows a cell with an elongated division site, leading to the formation of daughter cells with polar nubs or extensions. No stalks were observed on cells of strain CB15N Δ*popZ*. Scale bar, 1 μm.

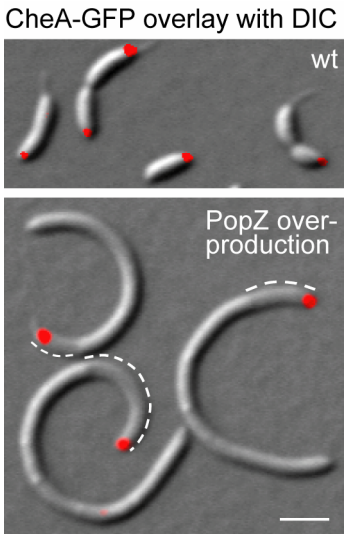

Figure S4. CheA localizes at the polar tip of the PopZ-rich region of *popZ*-overexpressing cells. Cells of strain CB15N *cheA::cheA-gfp*/pJS14Pxyl-PopZ grown in PYE were treated with 0.3 % xylose for 6h in order to induce PopZ overproduction prior to fluorescence microscopy. Broken lines indicate PopZ-rich regions. Scale bar, 2 μm.

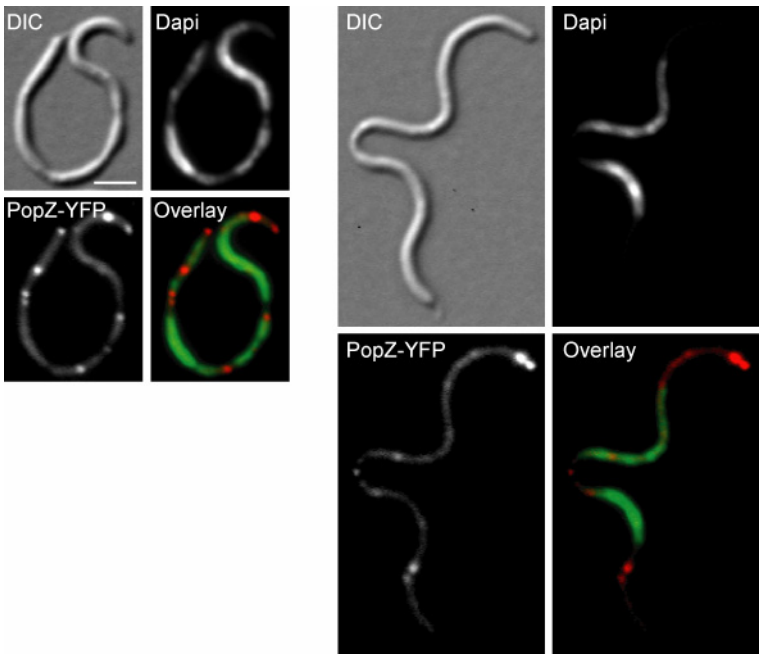

Figure S5. PopZ-YFP produced from the native promoter (as the only source of PopZ) forms multiple foci exclusively in chromosome-free regions.

Cells of strain CB15 *divD308(Ts)*::*pDW110 (parEp) divE309(Ts) popZ*::*pBGent-PopZ-YFP* were grown in liquid PYE medium at 30^{o}C and then shifted to the restrictive temperature 37^{o}C for 6h. PopZ-YFP is shown in red. Cells were stained with DAPI to visualize the DNA (green).

Supplemental Tables

Table S1. Localization of polar markers in wild-type and Δ*popZ* cells.

	TipN (%)		**PleC** (%)		**CheA** (%)		**CpaE** (%)		**FliG** (%)	
	wt	Δ*popZ*	wt	Δ*popZ*	wt	Δ*popZ*	wt	Δ*popZ*	wt	Δ*popZ*
Unipolar	71	8[b]	77	54	47	56[b]	68	55[b]	41	25[b]
Bipolar	10	87[c]	1	20	12	27[c]	5	10	48	71[c]
Nonpolar foci only	10[a]	3	0	5	0	7	0	2	0	2
Diffuse signal	9	2	22	21	41	10	30	33	11	2
Total cell count	318	203	359	258	303	220	321	214	270	231

Experiments were carried out using the following strains (grown in PYE at 30˚C). CB15N Δ*tipN*::*pHL32-1kb-tipN-gfp*, CB15N Δ*popZ* Δ*tipN*::*pHL23-1kb-tipN-gfp*, CB15N *pleC*::*pleC-yfp*, CB15N Δ*popZ pleC*::*pleC-yfp*, CB15N *cheA*::*cheA-gfp*, CB15N Δ*popZ cheA*::*cheA-gfp*, CB15N *cpaE*::*yfp-cpaE*, CB15N Δ*popZ cpaE*::*yfp-cpaE*, CB15N *xylX*::*Pxyl-fliG-gfp* and CB15N Δ*popZ xylX*::*Pxyl-fliG-gfp*

a) All cells had a single focus located at mid-cell

b) Includes cells with only one unipolar focus and cells with a unipolar focus and one or more additional internal foci.

c) Includes cells with only two foci (bipolar) and cells with two bipolar foci plus one or more additional internal foci.

Table S2: Strains and plasmids

Name	Relevant genotype/description	Reference
***C. crescentus* strains**		
CJW27	CB15N (also known as NA1000)	(Evinger and Agabian, 1977)
CJW686	CB15N *pleC*::*pleC-yfp*	(Matroule et al., 2004)
CJW826	CB15N *divJ*::*divJ-yfp*	(Lam et al., 2003)
CJW1329	CB15N *cckA*::*cckA-mgfp*	Peter Angelastro
CJW1407	CB15N Δ*tipN*	(Lam et al., 2006)
CJW1472	CB15N *xylX*::*pXGFP4*	Audrey Jackson
CJW2052	CB15N/pJS14Pxyl-PopZ	This work
CJW2053	CB15N Δ*tipN*::*pHL32-1kb-tipN-gfp*	Whitman Schofield
CJW2075	CB15N *cckA*::*cckA-mgfp*/pJS14Pxyl-PopZ	This work
CJW2214	CB15N Δ*popZ parB*::*cfp-parB xylX*::*pXmYFP4-PopZ*	This work
CJW2232	CB15N Δ*popZ cheA*::*cheA-gfp*	This work
CJW2234	CB15N *cheA*::*cheA-gfp*/pJS14Pxyl-PopZ	This work
CJW2237	CB15N *parB*::*cfp-parB popZ*::*pBGent-PopZ-YFP*	This work
CJW2238	CB15N Δ*popZ*	This work
CJW2239	CB15N *ftsZ*::*pBJM1 parB*::*cfp-parB*	This work
CJW2244	CB15N *popZ*::*pBGent-PopZ-TC*	This work
CJW2245	CB15N *popZ*::*pBGent-PopZ-YFP*	This work
CJW2246	CB15N/pJS14Pxyl-PopZ-TC	This work
CJW2249	CB15N Δ*popZ parB*::*cfp-parB*	This work
CJW2250	CB15N Δ*popZ divJ*::*divJ-yfp*	This work
CJW2251	CB15N Δ*popZ cckA*::*cckA-mgfp*	This work
CJW2252	CB15N Δ*popZ pleC*::*pleC-yfp*	This work
CJW2253	CB15N Δ*popZ cpaE*::*yfp-cpaE*	This work
CJW2254	CB15N *divJ*::*divJ-yfp*/pJS14Pxyl-PopZ	This work
CJW2255	CB15N *Cori*::*Cori-lacOp-kan xylX*::*pHPV472*/pJS14Pvan-PopZ	This work
CJW2256	CB15N/pJS14Pxyl-GFP-PopZ	This work
CJW2257	CB15N Δ*popZ xylX*::*Pxyl-fliG-gfp*	This work
CJW2259	CB15N Δ*tipN xylX*::*Pxyl-tipN*	Hubert Lam
CJW2260	CB15N Δ*popZ* Δ*tipN xylX*::*Pxyl-tipN*	This work
CJW2261	CB15N Δ*tipN xylX*::*pXmYFP4-popZ*	This work
CJW2262	CB15N Δ*popZ* Δ*tipN*::*pHL23-1kb-tipN-gfp*	This work
CJW2263	CB15N Δ*popZ mipZ*::*mipZ-yfp*	This work
CJW2264	CB15N Δ*popZ vanA*::*pMT400*	This work
CJW2265	CB15N *ftsZ*::*pBJM1 popZ*::*pBGentT-PopZ-YFP*	This work
CJW2266	CB15N *xylX*::*pXmYFP-PopZ*	This work
CJW2267	CB15N *xylX*::*pXGFP4*/pJS14Pvan-PopZ	This work
CJW2268	CB15N *parB*::*cfp-parB* /pJS14Pxyl-PopZ	This work
CJW2271	CB15N *vanA*::*pMT400*/pJS14Pxyl-PopZ	This work
CJW2658	CB15N *mipZ*::*mipZ-yfp*/pJS14Pxyl-PopZ	This work
CJW2662	CB15N *xylX*::*pXGFP4-PopZ*	This work
CJW2663	CB15N xylX::pXGFP4-PopZ/pJS14TipN	This work
CJW2702	CB15 *divD308(Ts)*::*pDW110 (parEp) divE309(Ts) xylX*::*pXmYFP4-popZ*	This work
CJW2816	CB15 *divD308(Ts)*::*pDW110 (parEp) divE309(Ts) popZ*::*pBGent-PopZ-YFP*	This work
MT15	CB15N *Cori*::*Cori-lacOp-kan xylX*::*pHPV472*	(Viollier et al., 2004)
MT97	CB15N *mipZ*::*mipZ-yfp*	(Thanbichler and Shapiro, 2006)
MT190	CB15N *parB*::*cfp-parB*	(Thanbichler and Shapiro, 2006)
MT199	CB15N *vanA*::*pMT400*	(Thanbichler and Shapiro, 2006)
NR1212	CB15N *cheA*::*cheA-gfp*	(Huitema et al., 2006)
NR1744	CB15N *xylX*::*Pxyl-fliG-gfp*	(Huitema et al., 2006)
PC6340	CB15 *divD308(Ts)*::*pDW110 (parEp) divE309(Ts)*	(Ward and Newton 1997)

PC7070	CB15N *recA*::Tn*5*	(Marczynski, 1999)
PV418	CB15N *cpaE*::*yfp-cpaE*	(Viollier et al., 2002)
YB1585	CB15N *ftsZ*::*pBJM1*	(Wang et al., 2001)
***E. coli* strains**		
CJW2222	MC1000/pNDM220CFP-ParB/pBAD33mYFP-PopZ	This work
CJW2226	MC1000/pNDM220CFP-ParB	This work
CJW2227	MC1000/pNDM220CFP-ParB/pBAD33PopZ-TC	This work
CJW2228	MC1000/pNDM220CFPMipZ/pBAD33PopZ-TC	This work
CJW2269	MC1000/pBAD33PopZ-TC	This work
CJW2270	MC1000/pBAD33mYFP-PopZ	This work
CJW2272	MC1000/pNDM220CFP-MipZ	This work
CJW2564	BL21/pET28aPopZ	This work
CJW2598	BL21/pET28aParB	This work
DH5α	Cloning strain	Invitrogen
MC1000	F- araD139 Δ(ara,leu)7697 Δ(lac)chi74 galU- galK- rpsL	(Casadaban and Cohen, 1980)
BL21	High-efficiency protein expression strain	Promega
S17-1	M294::RP4-2 (Tc::Mu)(Km::Tn7); for plasmid mobilization	(Simon et al., 1983)
Plasmids		
pBAD33	Medium copy number plasmid carrying the arabinose inducible P_{BAD} promoter, Chl^R	(Guzman et al., 1995)
pBAD33CFP-MipZ	pBAD33 carrying *cfp-mipZ* under control of P_{BAD}	This work
pBAD33CFP-ParB	pBAD33 carrying *cfp-parB* under control of P_{BAD}	This work
pBAD33mYFP-PopZ	pBAD33 carrying *myfp-popZ* under control of P_{BAD}	This work
pBAD33PopZ-TC	pBAD33 carrying *popZ-tc* under control of P_{BAD}	This work
pBGent	$Gent^R$ variant of pBGST18 integration vector	(Matroule et al., 2004)
pBGent-PopZ-TC	Integration plasmid carrying 3'-end of *popZ-tc*	This work
pBGent-PopZ-YFP	Integration plasmid with 3'-end of *popZ* fused to *yfp*	This work
pET28a	Expression vector used for N-term His-tag of PopZ and ParB	Novagen
pET28aPopZ	Plasmid expressing His_6-PopZ	This work
pET28aParB	Plasmid expressing His_6-ParB	This work
pEYFP-N1	Cloning vector carrying *yfp*	Clontech
pJS14	Medium copy number pBBR1-derived broad host range vector, Chl^R	Jeff Skerker
pJS14Pvan-PopZ	pJS14 carrying *popZ* under control of the vanillic acid inducible P_{van} promoter	This work
pJS14Pxyl-GFP-PopZ	pJS14 carrying *gfp-popZ* under control of the xylose inducible P_{xyl} promoter	This work
pJS14Pxyl-PopZ	pJS14 carrying *popZ* under control of P_{xyl}	This work
pJS14Pxyl-PopZ-TC	pJS14 carrying *popZ-tc* under control of P_{xyl}	This work
pJS14TipN	pJS14 carrying TipN under control of the native promoter	Hubert Lam
pKS-mYFP1	pKS derivative carrying *myfp*	Jean-Yves Matroule
pMR20	Low copy number broad host range vector, Tet^R	(Roberts et al., 1996)
pMR20Pxyl-PopZ	pMR20 carrying *popZ* under control of P_{xyl}	This work
pMT374	RK2 derivative that carries the P_{van} promoter	Martin Thanbichler
pNDM220	Low copy number temperature sensitive mini-R1 plasmid carrying the IPTG inducible $P_{A1/O4/O3}$ promoter, $lacIq^+$, Amp^R	(Gotfredsen and Gerdes, 1998)
pNDM220CFP-MipZ	pNDM220 carrying *cfp-mipZ* under control of $P_{A1/O4/O3}$	This work
pNDM220CFP-ParB	pNDM220 carrying *cfp-parB* under control of $P_{A1/O4/O3}$	This work
pNPTS138	Litmus 38-derived integration vector: $oriT^+$ $sacB^+$ Kan^R	MRK Alley
pNPTS-eCFP-ParB	pNPTS138 derivative carrying *ecfp* fused to the 5'-end of *parB*	(Thanbichler and Shapiro, 2006)
pNPTS138-PopZ::omega	$sacB^+$ integration vector with sequences up- and down-stream of *popZ*	This work
pRW431	Plasmid used for cloning of genes under control of P_{xyl}	Rachel Wright
pRW431-PopZ-TC	pRW431 carrying *popZ-tc* under control of P_{xyl}	This work
pXGFP4-C1	Integration vector carrying *gfp* under control of the P_{xyl}	MRK Alley
pXGFP4-PopZ	Integration plasmid carrying *gfp-popZ* under control of P_{xyl}	This work
pXmYFP4-PopZ	Integration plasmid carrying *myfp-popZ* under control of P_{xyl}	This work

Supplemental Experimental Procedures

Genomic overproduction screen

Genomic DNA of wild-type CB15N was digested with PstI, XhoI, XmaI or SacI. DNA fragments (3 to 7 kbp) were purified from agarose gels and ligated into medium copy plasmid pJS14 (chloramphenicol resistant, chl^R). The ligation mixtures were electroporated into CB15N *recA*::Tn*5*, which were then plated on PYE+chl to obtain about 200-300 colonies per plate. After 4 days at 30^{o}C, the smallest colonies (10%) were visually selected and grown at 30^{o}C in 96-well plates in liquid PYE (+chl) overnight. Cultures were then examined by microscopy to identify strains with cell division and/or branching phenotypes, which were collected for plasmid preparation, retransformation and sequencing. Sixty-five plasmids caused a cell division phenotype, which was confirmed by plasmid isolation and retransformation. Plasmid insert typically contained one or two chromosomal fragments coding for a total of 2 to 8 genes.

Construction of plasmids

pJS14Pxyl-PopZ: The *popZ* ORF was PCR amplified from a genomic DNA preparation of strain CB15N by using primers CC1319-1 and CC1319-2. The purified PCR product was digested with NdeI and KpnI. An EcoRI-NdeI fragment containing the xylose inducible promoter (*Pxyl*) was cut out from plasmid pRW431. The two fragments were ligated into the EcoRI-KpnI sites of vector pJS14.

pRW431-PopZ-TC: *popZ* was PCR amplified from a genomic DNA preparation of strain CB15N by using primers CC1319-1 and CC1319-cTC (an 18bp sequence coding for the TC-tag was included in the primer, immediately before the *popZ* stop codon). The purified PCR product was

digested with NdeI and KpnI and ligated into the same sites of vector pRW431 (downstream of the xylose promoter).

pJS14Pyl-PopZ-TC: An EcoRI-KpnI fragment containing the xylose promoter and *popZ-tc* was cut out from plasmid pRW431-PopZ-TC. The fragment was ligated into the EcoRI-KpnI sites of vector pJS14.

pJS14Pvan-PopZ: The *popZ* sequence was cut out from plasmid pJS14Pxyl-PopZ by using restriction enzymes NdeI and KpnI. A HindIII-NdeI fragment containing the vanillic acid promoter was cut out from plasmid pMT374. The two fragments were ligated into the HindIII-KpnI sites of vector pJS14.

pJS14Pxyl-GFP-PopZ: A NdeI-KpnI fragment containing *gfp-popZ* was cut out of plasmid pXGFP4-PopZ. This fragment was cloned into the NdeI-KpnI sites of pJS14Pxyl-PopZ thereby replacing *popZ* with *gfp-popZ*.

pMR20Pxyl-PopZ: An EcoRI-KpnI fragment containing *Pxyl-popZ* was cut out of plasmid pJS14Pxyl-PopZ. This fragment was cloned into the same sites of vector pMR20.

pXGFP4-PopZ: *popZ* was PCR amplified from a genomic DNA preparation of strain CB15N by using primers CC1319-GFP3 and CC1319-2. The purified PCR product was digested with EcoRI and KpnI and ligated into vector pXGFP4-C1 to create a fusion of GFP to the N-terminus of PopZ.

pXmYFP4-PopZ: *myfp* was amplified from vector pKS-mYFP by using primers CFP1 and CFP2. The PCR product was digested with NdeI and *Eco*RI and ligated into the same sites of plasmid pXGFP4-PopZ thereby replacing *gfp* with *myfp*.

pNPTS138-PopZ::omega: ~600bp upstream of *popZ* and ~600bp downstream of *popZ* were PCR amplified from a genomic DNA preparation of strain CB15N by using primers CC1319-up1 + CC1319-up2 and CC1319-down3 + CC1319-down4, respectively. The upstream PCR fragment was digested with PstI and EcoRI. The downstream PCR fragment was digested with EcoRI and BsrGI. The two fragments were ligated into pNPTS138 cut with PstI and BsrGI, creating plasmid pNPTS138-PopZ-up-down. This plasmid was digested with EcoRI and ligated with an EcoRI fragment from plasmid pBOR containing the omega cassette.

pBGent-PopZ-TC: pRW431-PopZ-TC was digested with SacII, treated with Klenow polymerase and digested with HindIII. The fragment containing the 3'-end 186bp of *popZ* and the TC-tag was purified and ligated into vector pBGent digested with NdeI, treated with Klenow polymerase and digested with HindIII.

pBGent-PopZ-YFP: The last 284bp of *popZ* was PCR amplified from a chromosomal DNA preparation of CB15N by using primers CC1319-GFP8 and CC1319-GFP9. The purified PCR product was digested with HindIII and KpnI. *yfp* was PCR amplified from plasmid pEYFP-N1 by using primers YFP-1 and YFP-2. The PCR product was digested with restriction enzymes NdeI and KpnI. A triple ligation was performed with the two PCR products and vector pBGent digested with NdeI and HindIII.

pBAD33-mYFP-PopZ: *myfp-popZ* was PCR amplified from plasmid pXmYFP4-PopZ by using primers GFP-SD-1 (which contains an optimized *E. coli* Shine Dalgarno sequence upstream of the *myfp* start codon) and CC1319-2. The purified PCR product was cloned into SmaI and PstI of vector pBAD33.

pBAD33-PopZ-TC: *popZ* was PCR amplified from a genomic DNA preparation of CB15N by using primers 1319-SD-1 and 1319-TC2 (the 18bp sequence coding for the TC-tag was included in the primer, before the *popZ* stop codon). The purified PCR product was digested with restriction enzymes SmaI and PstI and ligated into the same sites of vector pBAD33.

pBAD33-CFP-ParB: A fragment containing *cfp* and the first 339bp of *parB* was PCR amplified from plasmid pNPTS-CFP-ParB by using primers GFP-SD-1 and PB-1. The purified PCR product was cut with SacI. The remaining part of *parB* (from bp 340-885) was PCR amplified from a genomic DNA preparation of strain CB15N by using primers PB-2 and PB-3. This PCR product was digested with SacI and HindIII. A triple ligation was performed using the two digested PCR products and vector pBAD33 digested with SmaI and HindIII.

pNDM220-CFP-ParB: *cfp-parB* was PCR amplified from plasmid pBAD33-CFP-ParB by using primer GFP-SD-1 and PB-3. The resulting PCR product was cloned into vector pNDM220 digested with XhoI and treated with Klenow. Clones carrying *cfp-parB* in the correct orientation after the IPTG inducible $P_{A1/O4/O3}$ promoter were found by performing test digests.

pBAD33-CFP-MipZ: *mipZ* was PCR amplified from a genomic DNA preparation of CB15N by using primers MZ1 and MZ2. The purified PCR product was digested with HindIII and ligated into pBAD33-CFP-ParB digested with HindIII, thereby replacing *parB* with *mipZ*. Clones carrying *mipZ* in the correct orientation were found by performing colony PCR.

pNDM220-CFP-MipZ: A SmaI-EcoRI fragment containing *cfp-mipZ* from plasmid pBAD33-CFP-MipZ was ligated into vector pNDM220 that had been digested with XhoI, treated with Klenow polymerase and digested with EcoRI.

pET28a-ParB:

parB was PCR amplified from a genomic DNA preparation of CB15N by using primers His-ParB-up and PB3. The purified PCR product was digested with NdeI and EcoRI and ligated into pET28a digested with the same enzymes.

pET28a-PopZ:

popZ was PCR amplified from a genomic DNA preparation of CB15N by using primers CC1319-1 and CC1319EcoRI. The purified PCR product was digested with NdeI and EcoRI and ligated into pET28a digested with the same enzymes.

List of primers:

Restriction enzyme recognition sites added to the primer sequence is shown in brackets after the primer name. Primers are listed in the 5' to 3' direction.

CC1319-1 (NdeI): CCCCCCATATGTCCGATCAGTCTCAAGAACC

CC1319-2 (KpnI): CCCCCGGTACCTTAGGCGCCGCGTCCCCGAG

CC1319-cTC (KpnI):

CCCCCGGTACCTTAACAACATCCTGGACAACAGGCGCCGCGTCCCCGAGAGATACG

CC1319-up1 (PstI): CCCCCCTGCAGGGCTTGACCACGTCGCAGGTCC

CC1319-up2 (EcoRI): CCCCCGAATTCCGTCGTAAAGAGGTACGAATCC

CC1319-down3 (EcoRI): CCCCCGAATTCGCCTAAAACTTCCGAACCGTCGG

CC1319-down4 (BsrGI): CCCCCTGTACAGTAGAGTTGGACGAACACCTTGCG

CC1319-GFP3 (EcoRI): CCCCCGAATTCTATGTCCGATCAGTCTCAAGAACC

CFP-1 (NdeI): CCCCCCCATATGGTGAGCAAGGGCGAGGAGC

CFP-2 (EcoRI-HindIII): CCCCCGAATTCGAAGCTTGCTTGTACAGCTCGTCCATGCCG

GFP-SD-1 (SmaI):

CCCCCCCGGGAATAAGGAGGATTTACATATGGTGAGCAAGGGCGAGG

CC1319-2 (PstI):CCCCCCTGCAGTTAGGCGCCGCGTCCCCGAG

1319-SD-1 (SmaI):

CCCCCCCGGGAATAAGGAGGATTTACATATGTCCGATCAGTCTCAAGAACC

1319-TC2 (PstI):

CCCCCCTGCAGTTAACAACATCCTGGACAACAGGCGCCGCGTCCCCGAGAGATACG

YFP-1 (KpnI): CCCCCCGGTACCATGGTGAGCAAGGGCGAGGAGC

YFP-2 (SmaI-NdeI): CCCCCCCGGGCATATGTTACTTGTACAGCTCGTCCATGCCG

CC1319-GFP8 (HindIII): CCCCCAAGCTTGAGTCGGAACCGGCCTACACGC

CC1319-GFP9 (KpnI): CCCCCGGTACCGGCGCCGCGTCCCCGAGAGATACG

PB1 (SacI): CCCCCGAGCTCGCGCACCATGATCGG

PB2 (SacI): CCCCCGAGCTCGACGACCTGGCGGTGC

PB3 (EcoRI-HindIII): CCCCCGAATTCAAGCTTTCAGATCCCGCGCGTCAGTCG

MZ1 (HindIII): CCCCCAAGCTTCGATGGCCGAAACGCGCGTTATCG

MZ2 (HindIII-EcoRI): CCCCCAAGCTTGAATTCTTACTGCGCCGCCAGCATCG

His-ParB-up (NdeI): CCCCCCATATGTCCGAAGGGCGTCGTGGTCTGG

CC1319-EcoRI (EcoRI): CCCCCGAATTCTTAGGCGCCGCGTCCCCGAG

Construction of strains

CJW2238: A strain carrying a deletion of *popZ* was created by means of a two-step gene disruption method and sucrose selection (Gay et al., 1985). First, plasmid pNPTS138-PopZ::omega was electroporated into strain CB15N, selecting for kanamycin resistance (1. cross-over step). Next, a second cross-over step was performed in both the absence and presence of a complementing plasmid, pMR20Pxyl-PopZ, from which *popZ* expression could be induced by addition of xylose. This was done in order to test whether *popZ* might be essential for viability. Overnight cultures of strains CB15N *popZ*::*pNPTS138 PopZ*::*omega* and CB15N *popZ*::*pNPTS138-PopZ*::*omega*/pMR20Pxyl-PopZ (grown with xylose) were plated on PYE plates containing 3% sucrose and strep/spec (and xylose or glucose for the strain carrying pMR20Pxyl-PopZ). Colonies that grew on sucrose and lost kanamycin resistance were tested by PCR to verify the deletion of *popZ*. A similar number of positive Δ*popZ* colonies were obtained on all plates, demonstrating that PopZ is not essential for viability. Consistent with *popZ* being non-essential, a ϕCR30 phage lysate prepared from strain CB15N Δ*popZ* yielded a similar number of colonies when transduced into strain CB15N or strain CB15N/pMR20Pxyl-PopZ (with or without xylose in the plates).

CJW2244 and CJW2245: Plasmid pBGent-PopZ-TC and pBGent-PopZ-YFP, respectively, were conjugated into strain CB15N. It should be noted that cells of strain CJW2244, expressing PopZ-TC as the only copy, look wild-type under all growth conditions indicating that PopZ-TC is fully functional. Cultures of strain CJW2245, expressing PopZ-YFP as the only copy, seem to contain some elongated cells when grown in PYE, whereas the majority of cells look wild-type when cells are grown in M2G.

CJW2816: Plasmid pBGent-PopZ-YFP was conjugated into strain CB15 *divD308(Ts)::pDW110 (parEp) divE309(Ts)*.

CJW2237 and CJW2265: A ϕCR30 phage lysate prepared from strain CB15N *popZ*::*pBGent-PopZ-YFP* was used to transduce strain CB15N *parB*::*cfp-parB* and CB15N *ftsZ*::*pBJM1*, respectively.

CJW2249, CJW2263 and CJW2264: A ϕCR30 phage lysate prepared from strain CB15N Δ*popZ* was used to transduce strain CB15N *parB*::*cfp-parB* (MT190), CB15N *mipZ*::*mipZ-yfp* (MT97) and CB15N *vanA*::*pMT400* (MT199), respectively.

CJW2239: A ϕCR30 phage lysate prepared from strain CB15N *ftsZ*::*pBJM1*was used to transduce strain CB15N *parB*::*cfp-parB*, selecting for kanamycin resistance.

CJW2266, CJW2214, CJW2261 and CJW2702: Plasmid pXmYFP4-PopZ was conjugated into strain CB15N (CJW27), CB15N Δ*popZ parB*::*cfp-parB* (CJW2249), CB15N Δ*tipN* (CJW1407) and

CB15 *divD308(Ts)*::*pDW110 (parEp) divE309(Ts)* (PC6340), respectively. Integration at the *xylX* locus was verified by performing colony PCR. Production of mYFP-PopZ from the *Pxyl* promoter (PYE + 0.03% xylose) fully complements a Δ*popZ* deletion based on cell morphology. This full complementation presumably requires the somewhat higher level of expression achieved by the *Pxyl* promoter because expression of the same N-terminal mYFP fusion from the native promoter gives rise to a heterogenous population of normal and elongated cells (data not shown).

CJW2662: Plasmid pXGFP4-PopZ was conjugated into strain CB15N (CJW27). Integration at the *xylX* locus was verified by performing colony PCR. The degree of functionality of GFP-PopZ appeared similar to that of mYFP-PopZ produced from strain CJW2266.

CJW2663: Plasmid pJS14TipN was conjugated into strain CB15N *xylX*::*pXGFP4-PopZ* (CJW2662).

CJW2251, CJW2250, CJW2252, CJW2253, CJW2232 and CJW2257: A ϕCR30 phage lysate prepared from strain CB15N Δ*popZ* (carrying an omega –spec/strep^{R}- cassette in place of the *popZ* coding sequence) was used to transduce the Δ*popZ* mutation into strains CB15N *cckA*::*cckA-mgfp* (CJW1329), CB15N *divJ*::*divJ-yfp* (CJW826), CB15N *pleC*::*pleC-yfp* (CJW686), CB15N *cpaE*::*yfp-cpaE* (PV418), CB15N *cheA*::*cheA-gfp* (NR1212) and CB15N *xylX*::*xylX-fliG-gfp* (NR1744), respectively.

CJW2262 and CJW2260: A ϕCR30 phage lysate prepared from strain CB15N Δ*popZ* was used to transduce the Δ*popZ* mutation into strains CB15N Δ*tipN*::*pHL23-1kb-tipN-gfp* (CJW2053) and CB15N Δ*tipN xylX*::*Pxyl-tipN* (CJW2259), respectively.

CJW2052, CJW2268, CJW2271, CJW2254, CJW2075, CJW2234 and CJW2658: Plasmid pJS14Pxyl-PopZ was conjugated into strains CB15N (CJW27), CB15N *parB*::*cfp-parB* (MT190), CB15N *vanA*::*pMT400* (MT199), CB15N *divJ*::*divJ-yfp* (CJW826), CB15N *cckA*::*cckA-mgfp* (CJW1329), CB15N *cheA*::*cheA-gfp* (NR1212) and CB15N *mipZ*::*mipZ-gfp* (MT97), respectively.

CJW2246: Plasmid pJS14Pxyl-PopZ-TC was conjugated into strain CB15N (CJW27).

CJW2256: pJS14Pxyl-GFP-PopZ was conjugated into strain CB15N (CJW27).

CJW2267 and CJW2255: pJS14Pvan-PopZ was conjugated into strain CB15N *xylX*::*pXGFP4* (CJW1472) and CB15N *Cori*::*Cori-lacOp-kan xylX*::*pHPV472* (MT15), respectively.

CJW2269, CJW2270, CJW2226, CJW2272, CJW2227, CJW2228 and CJW2222: *E. coli* strains were prepared by electroporation or transformation of the relevant plasmids into strain MC1000.

CJW2564 and CJW2598: pET28a-PopZ and pET28a-ParB, respectively, were electroporated into strain BL21.

Supplemental References

Briegel, A., Dias, D. P., Li, Z., Jensen, R. B., Frangakis, A. S., and Jensen, G. J. (2006). Multiple large filament bundles observed in *Caulobacter crescentus* by electron cryotomography. Mol Microbiol *62*, 5-14.

Casadaban, M. J., and Cohen, S. N. (1980). Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J Mol Biol *138*, 179-207.

Evinger, M., and Agabian, N. (1977). Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J Bacteriol *132*, 294-301.

Gay, P., Le Coq, D., Steinmetz, M., Berkelman, T., and Kado, C. I. (1985). Positive selection procedure for entrapment of insertion sequence elements in gram-negative bacteria. J Bacteriol *164*, 918-921.

Gotfredsen, M., and Gerdes, K. (1998). The *Escherichia coli* relBE genes belong to a new toxin-antitoxin gene family. Mol Microbiol *29*, 1065-1076.

Guzman, L. M., Belin, D., Carson, M. J., and Beckwith, J. (1995). Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol *177*, 4121-4130.

Huitema, E., Pritchard, S., Matteson, D., Radhakrishnan, S. K., and Viollier, P. H. (2006). Bacterial birth scar proteins mark future flagellum assembly site. Cell *124*, 1025-1037.

Lam, H., Matroule, J. Y., and Jacobs-Wagner, C. (2003). The asymmetric spatial distribution of bacterial signal transduction proteins coordinates cell cycle events. Dev Cell *5*, 149-159.

Lam, H., Schofield, W. B., and Jacobs-Wagner, C. (2006). A landmark protein essential for establishing and perpetuating the polarity of a bacterial cell. Cell *124*, 1011-1023.

Marczynski, G. T. (1999). Chromosome methylation and measurement of faithful, once and only once per cell cycle chromosome replication in *Caulobacter crescentus*. J Bacteriol *181*, 1984-1993.

Matroule, J. Y., Lam, H., Burnette, D. T., and Jacobs-Wagner, C. (2004). Cytokinesis monitoring during development; rapid pole-to-pole shuttling of a signaling protein by localized kinase and phosphatase in *Caulobacter*. Cell *118*, 579-590.

Roberts, R. C., Toochinda, C., Avedissian, M., Baldini, R. L., Gomes, S. L., and Shapiro, L. (1996). Identification of a *Caulobacter crescentus* operon encoding *hrcA*, involved in negatively regulating heat-inducible transcription, and the chaperone gene *grpE*. J Bacteriol *178*, 1829-1841.

Simon, R., Prieffer, U., and Puhler, A. (1983). A broad host range mobilization system for *in vivo* genetic engineering: Transposon mutagenesis in gram negative bacteria. Biotechnology *1*, 784-790.

Thanbichler, M., and Shapiro, L. (2006). MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. Cell *126*, 147-162.

Viollier, P. H., Sternheim, N., and Shapiro, L. (2002). A dynamically localized histidine kinase controls the asymmetric distribution of polar pili proteins. EMBO J *21*, 4420-4428.

Viollier, P. H., Thanbichler, M., McGrath, P. T., West, L., Meewan, M., McAdams, H. H., and Shapiro, L. (2004). Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. Proc Natl Acad Sci U S A *101*, 9257-9262.

Wang, Y., Jones, B. D., and Brun, Y. V. (2001). A set of *ftsZ* mutants blocked at different stages of cell division in *Caulobacter*. Mol Microbiol *40*, 347-360.

Ward, D., and Newton, A. (1997). Requirement of topoisomerase IV parC and parE genes for cell cycle progression and developmental regulation in *Caulobacter crescentus*. Mol Microbiol *26*, 897-910.